The Interaction of *Fusarium oxysporum* and *Rhizoctonia solani* in Causing Root Rot of Soybeans

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This study was part of project 0346 of the Agricultural Experiment Station, College of Agriculture, University of Illinois, Urbana-Champaign. It was supported in part by a grant from Illinois Crop Improvement Association.

Accepted for publication 21 December 1987 (submitted for electronic processing).

ABSTRACT

Datnoff, L. E., and Sinclair, J. B. 1988. The interaction of *Fusarium oxysporum* and *Rhizoctonia solani* in causing root rot of soybeans. Phytopathology 78:771-777.

In the growth chamber, disease severity and incidence of soybean roots were greater in plants inoculated with *Fusarium oxysporum* and *Rhizoctonia solani* than with either alone. Root infection and recovery of *R. solani* were lower in the presence of *F. oxysporum*, and greater for *F. oxysporum* in the presence of *R. solani* than for either pathogen alone. In the field, root infection did not differ significantly ($P = 0.05$) when plants were inoculated with both fungi or by *R. solani* alone. Disease-severity ratings for plants in plots infested with the pathogen mixture also were greater than with either pathogen alone up to 36 days after planting. Plants inoculated with both fungi or with *R. solani* alone were shorter than plants uninoculated or inoculated with *F. oxysporum*. The interaction of *F. oxysporum* and *R. solani* in causing a root rot of soybean appears to be additive.

Root rots of crops may involve more than one pathogen (8,9,29). The disease caused by two or more pathogens often are more severe than that caused by a single pathogen (10). These interactions influence disease incidence and severity and plant growth (28,32).

Fusarium oxysporum Schlect. emend. Snyd. & Hans. and *Rhizoctonia solani* Kühn cause root rots in soybean (*Glycine max* (L.) Merr.) wherever the crop is grown (30). Most reports show disease caused by one or the other pathogen (18). However, when Cromwell (5) first reported *Fusarium* blight of soybeans, he noted that *R. solani* also affected these plants in the same field. French and Kennedy (12) isolated both *F. oxysporum* and *R. solani* from soybean plants in the same field and suggested that *F. oxysporum* was either a secondary invader or co-invader with *R. solani*. In Brazil, both fungi were isolated from soybean plants affected by dead patch disease (2,22,24).

We investigated the interaction of *F. oxysporum* and *R. solani* in causing a root rot of soybeans by determining the effects of inoculum densities of these two pathogens on root infection, disease incidence, disease severity, and plant growth of soybeans in growth chambers and field plots. A portion of this work has been reported (7).

MATERIALS AND METHODS

Inoculum preparation. Cultures of *F. oxysporum* were obtained from R. B. Carroll (Department of Plant Sciences, University of Delaware). Speciation was determined by using carnation-leaf agar and potato-dextrose agar (PDA) slants, as recommended by the Fusarium Research Center, Pennsylvania State University, College Park (26). Single-spored cultures were isolated and maintained in sterile soil at 4 C. Culture identification was confirmed at the Fusarium Research Center.

Cultures of a multinucleate *R. solani* were obtained from J. E. Bowman (Department of Plant Pathology, University of Illinois, Urbana-Champaign) and maintained on acidified (pH 4.3) PDA (Difco) at 4 C. Identification was confirmed based on microscopic characters (27). Inoculum was grown in 500 ml of yeast-dextrose broth (5 g of yeast extract, 5 g of peptone, 20 g/L of dextrose) in Fernbach flasks. The medium was seeded by placing a 4-day-old hyphal tip from the margin of a fresh culture on a sterile cork resting on top of the broth (13). Mycelia grew radially over the medium, forming a mycelial mat. After 10–14 days at 25 C, the mycelial mats (approximately 50 g) were harvested. They were rinsed with sterile deionized distilled water, blotted dry, and minced in a Waring Blendor in 10 ml of sterile, deionized water for 15–30 sec.

Production of soybean test seedlings. Seeds of soybean cultivar Wells II were surface-disinfested in 0.5% NaOCl for 3.5 min, followed by three 1-min rinses in sterile deionized distilled water. Seeds were planted in flats of sterile vermiculite in the greenhouse or laboratory, and seedlings were removed after 3 days for growth-chamber studies and after 5 days for field studies.

Soil mixture, infestation, and moisture. Soil (Drummer silt loam) and sand were sieved through a 2-mm-mesh sieve and mixed in equal volumes in a cement mixer, steamed for 4 hr, cooled for 24 hr, and steamed again for 4 hr. After 48 hr, the mixture of sand and soil was infested with either *F. oxysporum* or *R. solani* alone or in combination. In growth-chamber studies, conidia of *F. oxysporum* per gram of soil were applied by atomization to the soil mixture contained in polyethylene bags. *R. solani* was applied to the soil mixture based on gram of wet-weight mycelium per gram of soil. These artificially infested soil samples subsequently were mixed by hand. For field studies, conidia of *F. oxysporum* and mycelia of *R. solani* were applied to the soil similarly but mixed in a cart cement mixer for 20 min. Soil moisture content was determined gravimetrically (16).

Estimation of inoculum concentrations. Propagule density of *F. oxysporum* in infested soil was determined on Komada's *Fusarium*-selective medium (20) with dilution plates (25). Propagule density of *R. solani* was determined by using a soil pellet sampler (15) and was expressed as the number of hyphae emerging from one center in each pellet on Ko's agar medium (19). For growth-chamber studies, inoculum densities of *F. oxysporum* ranged from 10^2 to 10^6 spores per gram of soil in 10-fold increments, whereas *R. solani* ranged from 0.02 to 0.08 propagules (gram of wet-weight mycelium per gram of soil). When inoculum density of *F. oxysporum* ranged from 10^2 to 10^6 conidia per gram of soil, *R. solani* was added at 0.04 g per gram of soil. When

R. solani ranged from 0.02 to 0.08 g per gram of soil, *F. oxysporum* was added at 1×10^6 conidia per gram of soil. For field studies, inoculum densities of *F. oxysporum* ranged from 1 to 2.4×10^6 conidia per gram of soil, and for *R. solani*, 0.04 gram of wet-weight mycelium per gram of soil.

Isolations from root tissues and percent root infections. The first 5-cm section of taproot below the soil line was cut into 1-cm sections, wrapped in two layers of cheesecloth, surface-disinfested for 3 min in 0.05% NaOCl, and then washed in tap water for 1–4 hr by utilizing a pipette washer. The root pieces were given three 1-min rinses in sterile deionized distilled water and were randomly selected before transfer onto water agar and/or PDA amended with 100 mg of either tetracycline or chloramphenicol, and Komada's *Fusarium*-selective medium. Each plate of selective medium contained five root pieces. All plates were incubated in continuous dark for 5–7 days at 25 C.

Root infection percentage was determined by counting the number of root pieces containing at least one colony of either fungus per plate, dividing by the total number of root pieces, and multiplying by 100 (infected root pieces ÷ total root pieces × 100). If a root piece contained more than one colony of either fungus, it was considered as one infected root piece.

Disease-severity rating and incidence. Soybean plants were rated for disease severity with a modification of the methods of Clarkson (4) and Lewis and Papavizas (23). Diseased plants were rated on a scale of 1–7, in which 1 = no symptoms; 2 = slight discoloration of the crown or several small lesions on the taproot or secondary roots; 3 = moderate discoloration with numerous small lesions on the crown or taproot or partial destruction of secondary roots; 4 = coalescing of lesions to form large lesions or some secondary roots destroyed; 5 = large lesions coalescing and extending into the cortex of the hypocotyl or taproot or both, some secondary roots destroyed; 6 = hypocotyl, crown, or taproot girdled and secondary roots destroyed; and 7 = death of the plant. Disease incidence was calculated as the proportion of diseased plants to the total number of plants.

Growth-chamber studies. Three-day-old Wells II soybean plants were transplanted into plastic tubes (Ray Leach Cone-tainers, Canby, OR) containing 160 ml of soil (pH 6.8–6.9), either uninfested or infested with *F. oxysporum* or *R. solani* alone or in

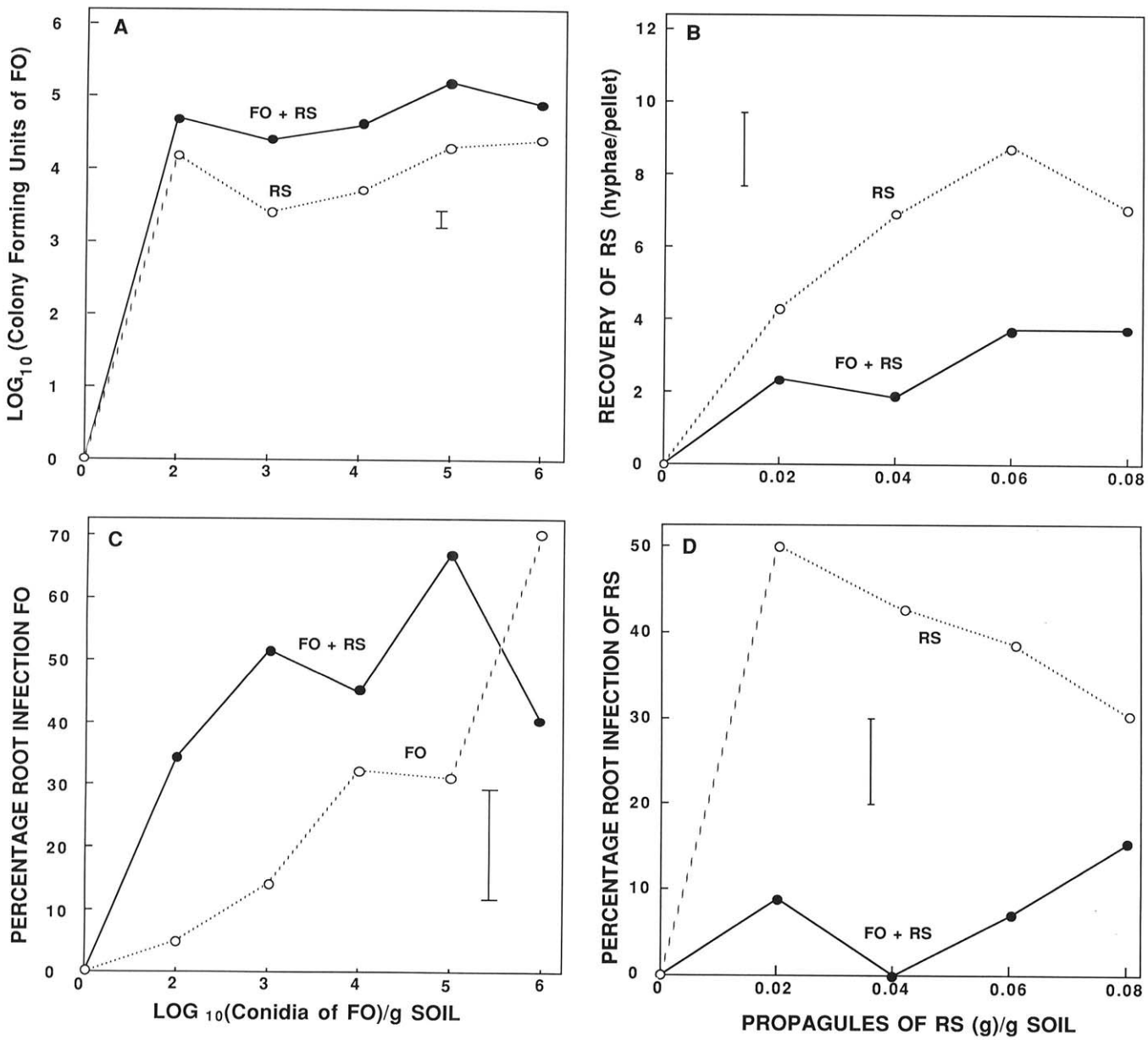

Fig. 1. The relationship of colony-forming units of *Fusarium oxysporum* (FO) **(A)** or recovery of *Rhizoctonia solani* (RS) **(B)** and percentage root infection of *F. oxysporum* **(C)** or *R. solani* **(D)** to increasing inoculum levels of *F. oxysporum* or *R. solani*. The vertical bar represents the least significant difference ($P = 0.05$).

combination as described previously. Plants in containers were placed in a growth chamber with 12 hr of light (240–310 $\mu E/m^2/sec$) at 24 ± 2 C. All plants were watered daily and fertilized weekly with 20 ml of a water-soluble fertilizer (Peter's 20-20-20). After 14 days, inoculum densities from bulked soil, root infection, disease severity, disease incidence, and dry weights of soybean plants were recorded.

Each treatment included three replications of 12 plants each arranged in a randomized complete block design. Data were analyzed as a 2 × 4 or 2 × 5 factorial. The two factors were the presence or absence of *F. oxysporum* or *R. solani*. The other factors were the incremental inoculum densities of the two pathogens. Each experiment was repeated at least once.

Field studies. During the 1982 and 1983 growing seasons, 5-day-old Wells II seedlings, at growth stage VC to V1 (11), were transplanted into 7.6 × 7.6-cm peat pots containing soil (pH 6.6) previously mixed with 100 g of controlled-release fertilizer (Osmocote 14-14-14, Sierra Chemical, Milpitas, CA) per 25 kg of soil. Before transplanting, the soil was either uninfested or infested with *F. oxysporum* or *R. solani* or with a mixture of both pathogens as described previously. All potted seedlings were planted in a field plot on the Agronomy-Plant Pathology South Farm, Urbana, 24 hr later. There were approximately 400 transplants per treatment. Transplants contained in peat pots were spaced about 3 cm apart. The nonpeat-pot controls also were transplanted, but were spaced much closer together. The plants were watered when needed.

At 14 and 22 days after planting, five plants and the artificially infested soil in peat pots were removed from each experimental unit with a hand spade. Propagule density, root infection, disease severity, disease incidence, plant dry weight, and height were recorded. Additional data sets were obtained at 14-day intervals until the R_6 growth stage (11).

The experimental design in the field was a split-plot in time. Main plots were the five treatments consisting of either pathogen alone, combinations of the pathogens, and controls with or without peat pots. Subplots were the sampling dates over the growing season. There were four and five replications of the treatments in 1982 and 1983, respectively.

Data were analyzed by the formula for least significant differences for split-plot (3) and randomized complete-block design (31) at $P = 0.05$.

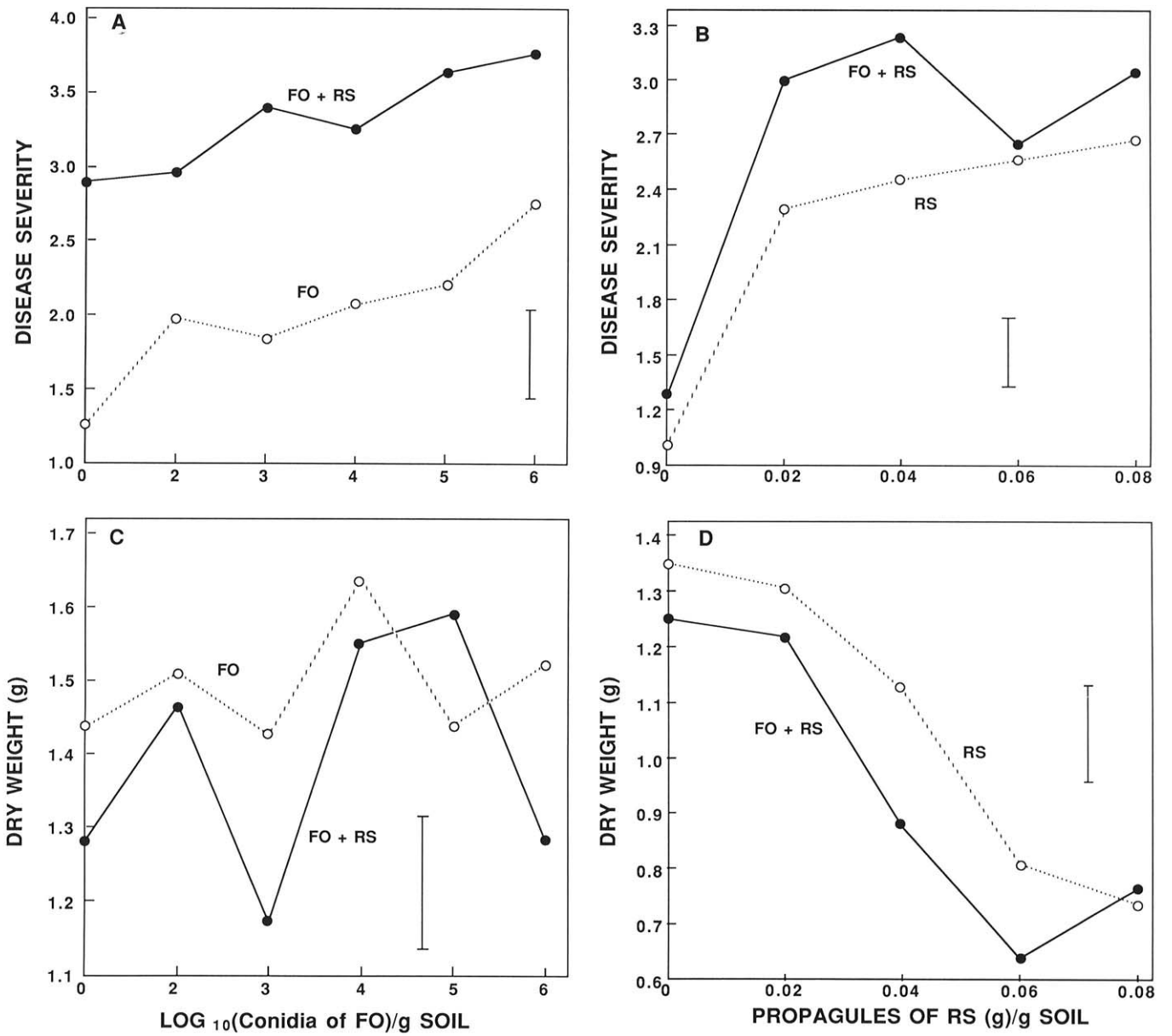

Fig. 2. The relationship of disease severity **(A, B)** and dry weight **(C, D)** of Wells II soybean plants to increasing inoculum levels of *F. oxysporum* (FO) or *R. solani* (RS). The vertical bar represents the least significant difference ($P = 0.05$).

RESULTS

Growth-chamber studies. The number of colony-forming units of *F. oxysporum* per gram of soil was greater in the presence of *R. solani* than with soil infested only with *F. oxysporum* (Fig. 1A). However, the number of hyphae of *R. solani* per soil pellet was significantly less in the presence of *F. oxysporum* than with *R. solani* alone at 0.04, 0.06, and 0.08 g of *R. solani* per gram of soil (Fig. 1B).

Root infection by *F. oxysporum* was generally greater in the presence of *R. solani* than by *F. oxysporum* alone (Fig. 1C) and was less only at the greatest density of *F. oxysporum* (1×10^6 conidia per gram of soil). However, root infection by *R. solani* was lower in the presence of *F. oxysporum* than when alone (Fig. 1D).

Disease severity for the pathogen mixture was significantly greater than for *F. oxysporum* alone or the control with increasing concentration of *F. oxysporum* (Fig. 2A). Disease severity for the pathogen mixture also was generally greater than for either pathogen alone and the control with increasing concentration of *R. solani* (Fig. 2B). Similar results also were observed for disease incidence (data not shown) (6).

Shoot dry weights did not differ significantly between the pathogen mixture and either pathogen alone or the controls with increasing concentration of *F. oxysporum* (Fig. 2C). The pathogen mixture and *R. solani* were significantly different in shoot dry weight compared with *F. oxysporum* alone and the controls at 0.04, 0.06, or 0.08 g of *R. solani* per gram of soil (Fig. 2D). The pathogen mixture only differed significantly from *R. solani* when mycelial concentration of *R. solani* was 0.04 g per gram of soil. Shoot dry weights decreased for plants inoculated with the pathogen mixture and for *R. solani* alone as the inoculum density of the latter increased (Fig. 2D).

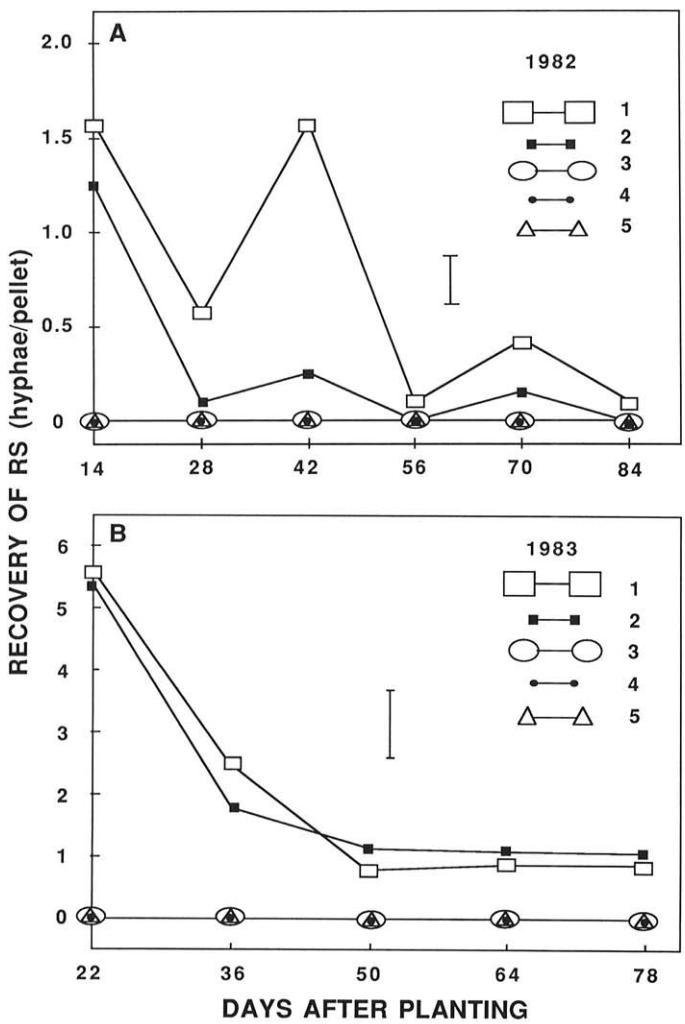

Fig. 3. The relationship of recovery of *R. solani* (RS) (number of hyphae per soil pellet) to days after planting for 1982 **(A)** and 1983 **(B)**. Treatments were: 1 = *F. oxysporum* (FO) + *R. solani* (RS); 2 = *R. solani* (RS); 3 = *F. oxysporum* (FO); 4 = peat-pot control (PPC); and 5 = without a peat-pot control (NPP). The vertical bar represents the least significant difference ($P = 0.05$).

Field studies. The recovery of *R. solani* from soil infested with both *R. solani* and *F. oxysporum* was lower than from soil infested only with *R. solani* at 28, 42, and 70 days after planting in 1982 (Fig. 3A). The recovery of *R. solani* did not differ between these two treatments in 1983 (Fig. 3B). No propagules of *R. solani* were detected in either year from treatments with no added inoculum of *R. solani*.

Root infection by *R. solani* generally was reduced in plots infested with both pathogens, although at 14 days (1982) and at 50 days (1983) after planting, there was greater infection by *R. solani* in the pathogen mixture (Fig. 4C and D). Root infection by *F. oxysporum* did not differ in the mixtures or in treatments infested only with *F. oxysporum* (Fig. 4A and B). In all treatments the amount of root infection by *F. oxysporum* from natural inoculum increased with time during both years.

The disease-severity ratings on plants in plots infested with the pathogen mixture or by *R. solani* alone differed from one another and from the other treatments up to 36 days after planting (Fig. 5A and B). Disease-severity ratings for plants in plots infested with *F. oxysporum* were significantly higher than controls up to 36 days. However, severity ratings declined in all treatments by the third recording date in both seasons. Similar results also were observed in both years for disease incidence (data not shown) (6).

Shoot dry weights of plants grown in plots infested by *R. solani* alone or the pathogen mixture were reduced from the controls, although they did not differ from each other (Tables 1 and 2). Less shoot dry-weight loss occurred in plots infested by *F. oxysporum* alone in both seasons. No consistent measurable dry-weight

TABLE 1. Effect of *Fusarium, Rhizoctonia,* or *Fusarium* and *Rhizoctonia* mixtures on the average dry weights (g) of Wells II soybeans sampled every 14 days after planting in 1982

	Days after planting					
Treatment[x]	14	28	42	56	70	84
FO + RS	5.23 a	10.53 a	29.32 a	94.07 ab	154.22 a	294.99 a
FO	5.40 ab	12.10 b	34.92 a	101.32 b	193.44 a	226.12 a
RS	5.45 b	10.95 a	32.02 a	79.39 a	174.30 a	225.54 a
PP	5.43 b	14.18 c	42.75 b	114.94 b	157.19 a	295.54 a
NPP	5.75 c	15.35 d	49.20 b	105.92 b	187.14 a	294.62 a
$FLSD_{0.05}$[y]	0.18	1.01	6.71	21.71	NS	NS
CV (%)[z]	2.20	5.60	11.57	14.22	···	···

[x]FO + RS = *Fusarium oxysporum* + *Rhizoctonia solani*; FO = *F. oxysporum*; RS = *R. solani*; PP = peat pot control; NPP = control without a peat pot.
[y]Fisher's least significant difference; NS = not significant.
[z]Coefficient of variation.

TABLE 2. Effect of *Fusarium, Rhizoctonia,* or *Fusarium* and *Rhizoctonia* mixtures on the average dry weights (g) of Wells II soybeans sampled every 14 days after planting, beginning at the 22nd day in 1983

	Days after planting				
Treatment[x]	22	36	50	64	78
FO + RS	0.17 a	3.84 a	42.64 a	145.46 ab	336.04 ab
FO	0.25 b	14.10 b	72.44 b	191.56 c	370.00 a
RS	0.19 a	5.68 a	42.20 a	147.96 ab	303.98 b
PP	0.32 c	16.80 b	73.04 b	162.94 bc	304.94 b
NPP	0.21 ab	9.00 c	39.08 a	127.64 a	176.58 c
$FLSD_{0.05}$[y]	0.05	3.01	9.07	32.89	46.84
CV (%)[z]	17.09	22.78	12.55	15.82	11.70

[x]FO + RS = *F. oxysporum* + *R. solani*; FO = *F. oxysporum*; RS = *R. solani*; PP = peat pot control; NPP = control without a peat pot.
[y]Fisher's least significant difference; NS = not significant.
[z]Coefficient of variation.

reduction occurred in any harvest beyond 56 days after planting.

Plant heights were reduced in plots infested with *R. solani* alone or the pathogen mixture, especially in midseason (Fig. 5C and D).

During the 1983 growing season, the control without peat pots had lower dry weights and plant heights compared with some of the other treatments (Table 2 and Fig. 5D). This reduction was caused by transplanting soybean seedlings too deep in the field. Although spacing of plants may have influenced dry weight and plant height, controls with and without peat pots were utilized to make relative comparisons to the other treatments.

DISCUSSION

The interaction of *F. oxysporum* and *R. solani* in causing a root rot of soybeans appears to be additive, because disease incidence and severity generally were greater for soybean plants in soil infested with the pathogen mixture than with either alone. Other investigators have demonstrated additive disease effects in soybeans or other crops when infected by more than one pathogen (1,14,17,29,32).

Powell (29) defined sequential disease complexes as those in which the primary pathogen infects and alters the host in advance of the subsequent secondary pathogen. The primary pathogen can induce disease whether or not the secondary pathogen becomes involved. These primary pathogens enter first, altering the host for colonization by the secondary pathogen. However, the secondary pathogen can alter the disease development and eventually may dominate the symptom syndrome of the disease complex.

Although we did not demonstrate if *R. solani* was the primary pathogen, our data suggested that this type of sequential disease complex was taking place. *F. oxysporum* tended to be isolated from soil and infected roots more frequently in the presence of *R. solani* than when alone. This also could account for the higher disease incidence and severity and the relatively lower dry weights of soybean seedlings inoculated with the pathogen mixture than with either fungus alone.

In 1983, we reported no apparent interaction between *F. oxysporum* and *R. solani* (7), although some competition or antagonism was suspected because propagule recovery of *R. solani* and root infection were lower in the presence of *F. oxysporum*. In

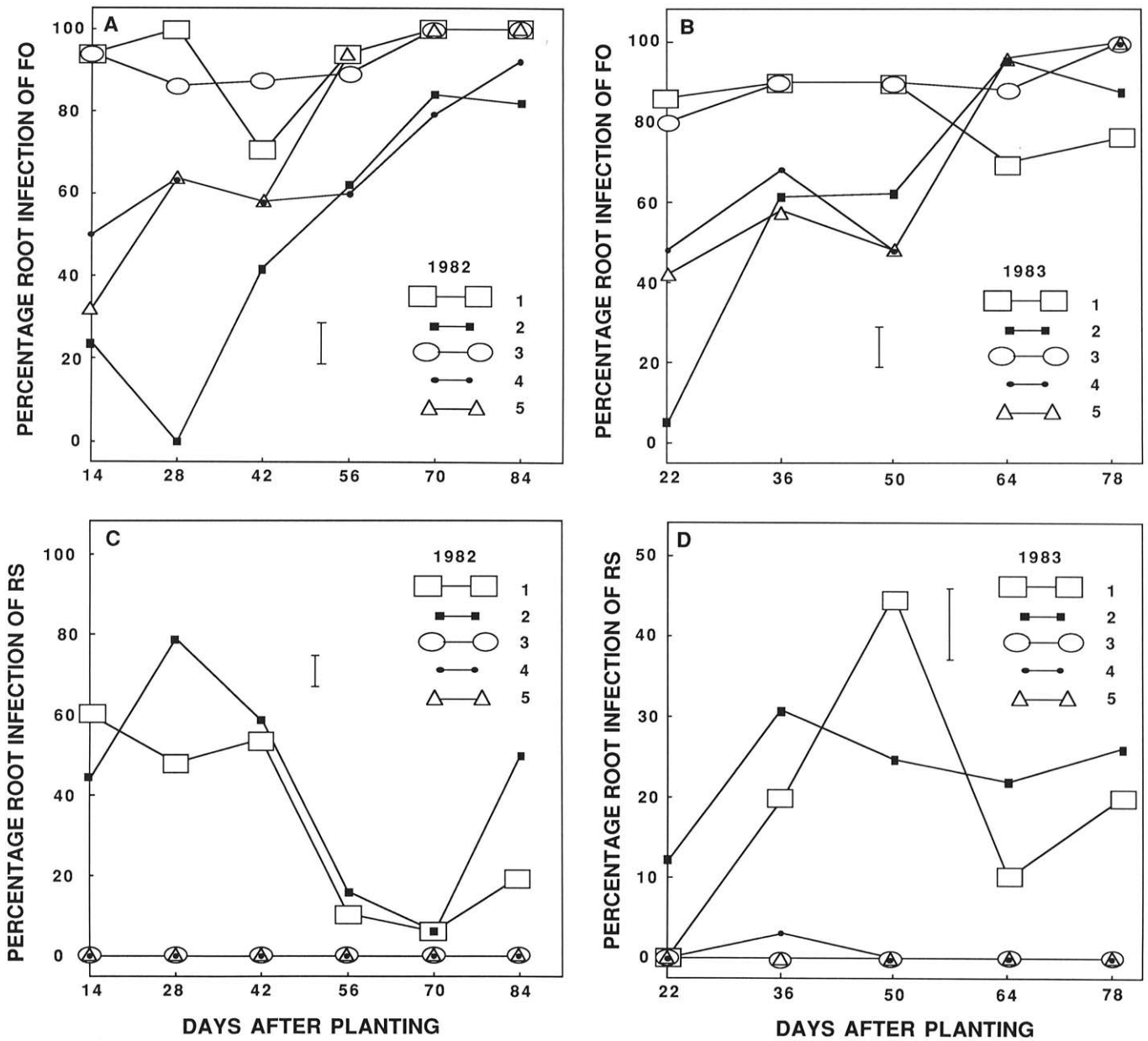

Fig. 4. The relationship of percentage root infection of *F. oxysporum* (FO) **(A, B)** or *R. solani* **(C, D)** (RS) to days after planting for 1982 and 1983. Treatments were: 1 = *F. oxysporum* (FO) + *R. solani* (RS); 2 = *R. solani* (RS); 3 = *F. oxysporum* (FO); 4 = peat-pot control (PPC); and 5 = without a peat-pot control (NPP). The vertical bar represents the least significant difference ($P = 0.05$).

addition, root infection and propagule recovery of *F. oxysporum* increased in the presence of *R. solani*. Although this same observation was not significantly obvious in the field during 1982 and 1983, root infection by *R. solani* generally was lower in the presence of *F. oxysporum* than alone. Evidently, *F. oxysporum* is competing with *R. solani* for infection sites, which could possibly explain the decrease in root infection by *R. solani*. Root infection by *R. solani* in the absence of *F. oxysporum* also decreased with increasing concentration of *R. solani* and was probably caused by the recolonization of steamed soil by airborne fungi such as *Trichoderma* and its subsequent antagonism of *R. solani*. Elarosi, in studying the synergistic relationship between *R. solani* and *F. solani* in causing a rot of potato tubers (9), noticed that *Fusarium* colonization, following infection by *Rhizoctonia*, will advance past it. This might explain why *F. oxysporum* was recovered more frequently than *R. solani* in our study. Kommedahl and Young (21) reported a significant decrease in the number of wheat seedlings infected by *R. solani* with increase in infected seedlings by *Fusarium* spp. when corn stalks were present in soil.

In a different pathogen combination, Zambolim et al (31) demonstrated that percentage root infection of soybeans was lower for *Macrophomina phaseolina* in combination with either *R. solani* or *F. solani* alone. However, percentage root infection with *F. solani* was higher in the presence of *M. phaseolina* than alone. Pieczarka and Abawi (28) reported no interaction between *R. solani* and *F. solani* f. sp. *phaseoli* on beans (*Phaseolus vulgaris*); however, the combination of both pathogens significantly reduced dry weight and plant height than either alone. Although root disease severity did not differ significantly between the pathogen mixture and *R. solani*, both differed from *F. s. phaseoli* alone. Similar observations were made under growth-chamber and field studies for our pathogen combination.

Other investigators have observed *F. oxysporum* and *R. solani* occurring together, but only in Brazil have the two pathogens been implicated in causing an interaction known as dead patch of soybeans. Both fungi were isolated and Koch's postulate demonstrated; however, *R. solani* was recovered more frequently,

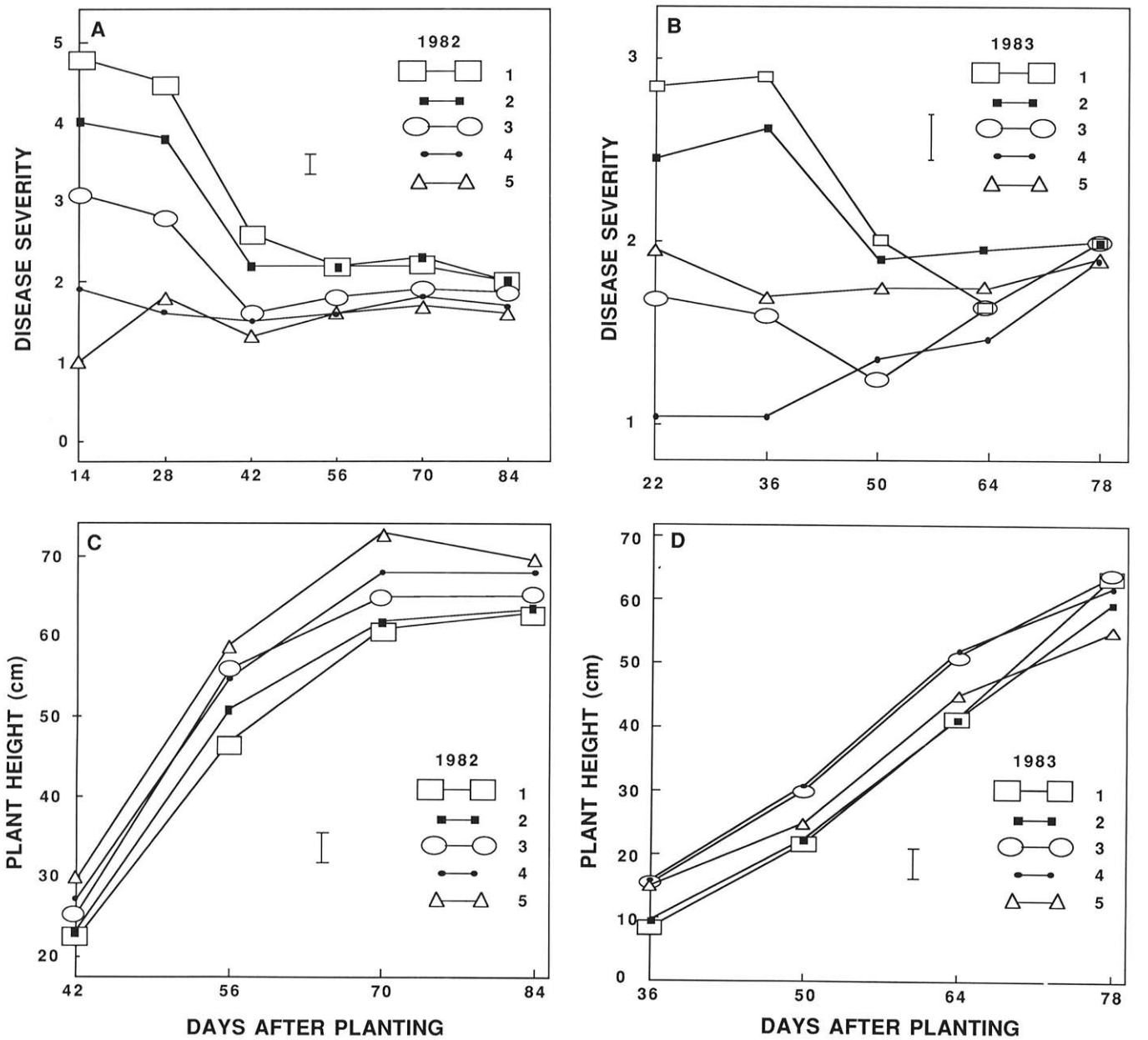

Fig. 5. The relationship of disease severity **(A, B)** and plant height **(C, D)** of Wells II soybeans planted in soil infested with *F. oxysporum* (FO) and/or *R. solani* (RS) to days after planting for 1982 and 1983. Disease severity rated on a scale of 1–7: 1 = healthy and 7 = dead. Treatments were: 1 = *F. oxysporum* (FO) + *R. solani* (RS); 2 = *R. solani* (RS); 3 = *F. oxysporum* (FO); 4 = peat-pot control (PPC); and 5 = without a peat-pot control (NPP). The vertical bar represents the least significant difference ($P = 0.05$).

especially during anthesis (22). Berton and Porto (2) also found that the pathogen mixture was slightly greater and lower for disease incidence and plant height, respectively, than *R. solani* alone, but only significantly different than *F. oxysporum* alone. These results support our studies. From these observations, *R. solani* was considered the principal causal agent of dead patch. This finding would help to support French and Kennedy's hypothesis that *F. oxysporum* is a secondary invader or co-invader with *R. solani* (12).

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